ImmPort Flow Cytometry Analysis Tool FAQ

1. **Why are there two versions of FLOCK?** ImmPort has two versions of the FLOCK algorithm; FLOCK v1 and FLOCK v2. The initial version of FLOCK, v1, supports the automatic identification of cell populations but requires defining appropriated bin and density threshold values for optimal results. Defining threshold values may be challenging—FLOCK v2 was developed to simplify the analysis process, reduce the need for defining thresholds and generates results similar to those achieved by manual gating without the time-consuming effort. Independent assessment of FLOCK v2 with other relevant methods will be released at FlowCAP: http://flowcap.flowsite.org. FLOCK v1 remains available to support existing analyses.
2. **Does the FLOCK algorithm perform compensation?** No. FLOCK is designed to allow the user to be freed from tedious and subjective gating procedures. While FLOCK may identify populations within uncompensated data, to ensure biological significance data should be compensated prior to uploading.
3. **Is there a limitation on the number of markers?** As the data dimension (i.e. number of markers) increases, there is a possibility that the current version of FLOCK will be unable to produce acceptable results due to the curse of dimensionality, however this will also be impacted by the number of events and the data distribution within the file. Experiments comprised of greater than 10 markers have been tested with satisfactory results. We encourage you to test your high dimensional data with FLOCK. Implementation of the next version of FLOCK will be geared toward addressing high dimensionality issues.
4. **What is FCS file conversion?** FCS (.fcs) files generated by instruments used in flow cytometry (FCM) experiments are in binary format and cannot be directly processed by independent analysis and visualization software. Reading and transforming a binary .fcs file requires understanding FCS file formats including FCS2.0 and FCS3.0. Unlike FCS2.0 which usually stores log-transformed data, FCS3.0 files keep the original raw outputs from the instrument in a linear mode. Analysis and visualization software needs to transform the linear-mode data before delivering the data to biologists. ImmPort employs FCSTrans, a flow cytometry data converter, which generates a data matrix output from FCS2.0 and FCS3.0 binary files. FCSTrans has been implemented in the ImmPort FCM data analysis pipeline—uploaded FCS files will be automatically converted and will be displayed as .txt converted files from View/Edit Uploaded Data found under Data Management in the analysis tool menu. For more information about

FCSTrans, please review the ImmPort FCS Conversion tutorial found under Help in the analysis tool menu.

5. **Which software should I use to convert .fcs to .txt?** For FCS v2 you can successfully use FCSExtract, flowCore in R, and FlowJo for MacOS (see conversion tutorials under Help). If you have FCS v3 we recommend FlowJo MacOS for the time being. While FCS v2 file conversion is consistent with the software noted, the same cannot be said for FCS v3. Conversion of .fcs files to .txt within the ImmPort flow cytometry module has been implemented and is automatic—there is no action required on the part of the user.
6. **Is there a limitation on the number of events FLOCK can handle?** No. The more events the file has, the longer you will need to wait for the results. For a file with 10,000 events, you will be able to visualize results within 1 minute. Files in excess of 10,000 events will take proportionally longer.
7. **What is a saved centroid file?** It is a file that records the centers of the populations identified by FLOCK. Centroids can be modified by the user within the ImmPort Flow Cytometry module via the View / Edit Centroid Results menu selection. The centroid file allows FLOCK to map populations across all samples so that the user can compare the same population in different samples.
8. **Can I batch upload my files into the Flow Cytometry modules for use with FLOCK?** Yes. Combining, or batching, files for upload has been implemented. Select Upload Multiple Files found under Data Management in the Flow Cytometry module menu bar. Batch uploads use the flowTextFiles.xls spreadsheet which allows the user to upload .fcs, .txt or both file types, edit marker names via the marker .info file, apply the same marker .info file to multiple files in the upload, change the name of uploaded files, provide informative descriptions to uploaded files and link uploaded .fcs and/or .txt files to previously existing experiment samples.
9. **Can I batch my files for FLOCK analysis?** Yes, once files are uploaded, you may select multiple files for FLOCK analysis. The files will be run sequentially and results can be viewed from View/Edit Results.
10. **What is required to analyze a sample with FLOCK?** FCS Files, either .fcs or .txt converted, are required to be uploaded into the ImmPort system. If .fcs files are uploaded, ImmPort automatically converts the files to .txt which is required for FLOCK analysis.
11. **How do I change the displayed marker names?** Go to Data Management from the menu and select View/Edit Uploaded Data. Select the file you wish to edit and click View Details. From the pop-up screen you will be able to edit marker names and save the changes.
12. **Can I change the population colors?** No. This is not currently implemented but is targeted for development.
13. **Can I compare two samples stained with different reagents**? No. Cross Sample Comparison is intended to compare only those samples stained with the same reagent panel.
14. **What results can be downloaded?** FLOCK results available for download include: population proportions, expression profiles, mean fluorescence

intensities as well as additional basic statistics about the FLOCK-defined populations. Mozilla Firefox users can right-click on FLOCK images and use the browser option "save image as" to copy images. Future development will target image downloads.

15. **Does FLOCK support histogram overlay?** No. This has been targeted for future development.
16. **How should I set FLOCK parameters?** If you would like to customize the parameter setting of FLOCK, you can begin with the automated identified parameter values and increase/decrease in subsequent analyses. Generally, increasing the number of bins increases the number of populations identified, while increasing the density threshold will decrease the number of populations identified.
17. **Can FLOCK results be opened by FlowJo?** No, but this has been targeted for future development.
18. **Does FLOCK provide a hierarchy of populations?** No. FLOCK directly identifies all cell populations within a sample. The user may utilize the expression profile generated by FLOCK to derive the hierarchical relationship among the populations within the sample. The expression profile is accessed by going to FLOCK from the View/Edit Results. Click Detail from the FLOCK Analysis History table, click Results and go to Summary Tables. Click Results to view the Summary table which displays expression profile as a range of values from 1-4.
19. **What is cell ontology and how is FLOCK related to cell ontology?** Cell ontology is a structured controlled vocabulary for cell types, which describes cell types and their phenotypes based on cell marker expression. A full description of the Cell Ontology can be found in Bard, Rhee and Ashburner. 2005. An Ontology for Cell Types. Cell populations identified by FLOCK are described with cell marker expression levels (see Results Summary Table) and are potentially interoperable among different flow cytometry data repositories.
20. **Is the FLOCK source code opensource?** Yes, the code can be found at http://immportflock.sourceforge.net